at page 38, line 28

after "...ATGCCACAAGTC", please insert --(SEQ ID No. 26)--

and at page 38, line 28 after "ATAGTCCTCGAGACTTTACTCTTCTGCTTC", please insert --(SEQ ID No. 27)--.

IN THE CLAIMS

Please amend claims as follows:

B1

14. **(Amended)** A substantially pure nucleic acid comprising a nucleotide sequence which encodes a CAK1 polypeptide at least 75% [homologous] identical to an amino acid sequence represented in SEQ ID No. 14.

B2

38.**(Amended)** The nucleic acid of claim 37, which nucleic acid encodes a *CAK1* polypeptide at least 75% [homologous] identical to an amino acid sequence represented in SEQ ID No. 14.

REMARKS

Applicants note that the Examiner has entered their preliminary amendments filed on 26 August, 1999. Applicants further note that the Examiner has acknowledged election, with traverse, of Group III, drawn to nucleic acids and their use in producing recombinant proteins. Applicants still further acknowledge the Examiner's reminder regarding the requirements for a formal Information Disclosure Statement under 37 CFR §1.98 (b). The Examiner's objections and rejections are discussed below.

Specification Objections

The specification has been objected to for failure to comply with 37 C.F.R. 1.821(d), which requires that a sequence listing identifier be used wherever reference is made to a nucleic acid or protein sequence. In particular, the Examiner cites the sequences on pages 35-38, which describe degenerate nucleic acid primer sequences utilized in the cloning of certain *Candida* genes. Accordingly, Applicants have amended the specification so as to include identifiers to additional listings of SEQ ID Nos. 15-27. In addition, a substitute sequence listing which

includes these additional SEQ ID Nos. is in preparation and, upon completion, will be forwarded as a supplemental amendment to this response. Applicant's respectfully request that the Examiner hold the requirement for a substitute sequence listing in abeyance until this replacement sequence listing is completed.

Claim Rejections under 35 U.S.C. §112

Claims 14-22 and 37-40 have been rejected under 35 U.S.C. §112, first paragraph. In particular, the Examiner states that: (a) "the specification does not reasonably provide the full scope of enablement for...a DNA encoding all variants and fragments of the CAK polypeptide which deviate to such an extent from the disclosed sequence as to be not functional"; and (b) "the specification does not reasonably provide the full scope of enablement for...any DNA wherein the encoded CAK polypeptide functions in one of either role of an agonist or an antagonist of cell cycle regulation of a *Candida* cell". Applicants respectfully traverse this rejection for the reasons set forth below.

35 U.S.C. §112, first paragraph enablement requirement

In subpart (a) of the Office Action, the Examiner states that "the experimentation necessary to make and use a DNA encoding CAK polypeptide which is 75% homologous with SEQ ID No. 14 is unpredictable". Applicants respectfully disagree. The "enablement" requirement of 35 U.S.C. §112, 1st paragraph requires that the specification of a patent must teach those skilled in the art at the time of the invention to make and use the full scope of the claimed invention without "undue experimentation". Applicants assert that these requirements are met in this instance.

First, the specification sets forth various protocols which could be used to identify the homologs of the CAK1-encoding genes in other animals. It is routine to use nucleic acid hybridization methods to obtain nucleic acids from other organisms which encode a polypeptide identical to or homologous with a given gene sequence from a particular organism. This approach to cloning homologs is discussed on page 13 (lines 16-26) of the specification. Furthermore, the specification provides numerous examples whereby *Candida* cell-cycle genes have been cloned using degenerate oligonucleotide primers (e.g. Example 1 beginning on page 35, which describes the cloning of the *Candida* TYP1 gene). The skilled artisan could apply these teachings to the

cloning of still other homologs of the disclosed genes, including the *CAK1* gene. Indeed, applicants note that the teachings in these examples and elsewhere in the specification are generally applicable to the cloning of homologs of the disclosed *Candid* cell-cycle genes. Accordingly, they support the cloning of the *Candida* Cdk-activating kinase (CAK) gene disclosed in the instant application as well as homologs of this gene in other organisms.

To further illustrate, the art is replete with examples of applications of PCR techniques to isolate and sequence DNA from distantly related species, including rare and even extinct organisms. In fact, Paabo and co-workers have successfully amplified sequences derived form a 7,000-year-old mummified brain and mitochondrial DNA using these techniques (See Paabo, S. et al. (1988) Nucl. Acid Res. 16(20):9775-87; Paabo, S. (1989) Proc. Natl. Acad. Sci USA 86: 1939-43). Moreover, the advent of PCR techniques has substantially simplified the cloning of new genes compared to conventional techniques. In fact, PCR techniques allow the cloning of unique sequences in a matter of hours (Saiki, R.K. et al. (1988) Science 239: 487-91). Moreover, the procedure is easily automated so that hundreds of samples can be amplified each day. Sequencing of the reaction products can be performed directly from the amplified product as described by Wrischnik, L.A. et al. (1987) Nucl. Acid Res. 15:529-42; Gyllenstein, U.B. and Erlich, H.A. (1988) Proc. Natl. Acad. Sci USA 85: 7652-56, which faciliates even further these procedures.

In addition, the specification is replete with examples of combinatorial techniques for identifying variants and fragments of naturally-occurring proteins which retain a particular biological activity of the naturally-occurring proteins, such as binding to a cyclin-dependent kinase. At the time of the instant invention, combinatorial techniques for generating and processing libraries of variants of a protein were routine in the art, even for libraries exceeding a billion different variants. For instance, those skilled in the art would recognize that claimed variants, including minimal binding domains of the subject CAK1 proteins, could be readily isolated by subjecting the protein to methods such as alanine scanning mutagenesis. See specification at pages 22-23. A review of the prior art demonstrates that such techniques were well within the purview of the skilled artisan. For example, alanine scanning mutagenesis is disclosed by (Lowman et al. (1991) Biochemistry 30:10832-10838; and Cunningham et al. (1989) Science 244:1081-1085). Examples of linker scanning mutagenesis may be found in Brown et al. (1992) Mol. Cell Biol. 12:2644-2652, and McKnight et al. (1982) Science 232:316. Similarly,

saturation mutagenesis is disclosed by Meyers et al. (1986) Science 232:613) and PCR mutagenesis is taught by Leung et al. (1989) Method Cell Mol Biol 1:11-19. Miller et al. (1992) A Short Course in Bacterial Genetics, CSHL Press, Cold Spring Harbor, NY disclose random mutagenesis.

Furthermore, the present application specifically teaches the use of such techniques, in the context of high throughput binding assays, in a manner which permits the isolation, from those libraries, of TYP1 polypeptides which retain certain bioactivities (see specification beginning at page 22, line 8). Applicants emphasize that the description of the generation of a TYP1 library was illustrative (page 8, line 13), and, by way of example, is applicable to CAK1 and the other genes disclosed in the application. Still further, the specification similarly discloses interaction trap assays which may be used to screen for CAK1 polypeptides which retain cyclin-dependent kinase binding activity (page 28, line 35 to page 29, line 30).

To further illustrate the state of the art, the Examiner's attention is directed to the review article of Gallop et al. (1994) J Med Chem 37:1233. Gallop et al., describes the general state of the art of combinatorial protein libraries throughout the early 1990's, e.g., before the filing date of the instant application. In particular, Gallop et al state at page 1239 "screening the analog libraries aids in determining the minimum size of the active sequence and in identifying those residues critical for binding and intolerant of substitution".

In addition, the Ladner et al. PCT publication WO90/02809, the Goeddel et al. U.S. Patent 5,223,408, and the Markland et al. PCT publication WO92/15679 illustrate specific techniques which one skilled in the art could utilize to generate libraries of CAK1 variants which can be rapidly screened to identify variants/fragments which retained a particular activity of the full-length CAK1 protein, e.g., such as binding to cyclin-dependent kinase. These techniques are exemplary of the art and demonstrate that large libraries of related variants/truncants can be generated and assayed to isolate particular variants without undue experimentation. The Examiner's attention is also directed to Gustin et al. (1993) Virology 193:653, and Bass et al. (1990) Proteins: Structure, Function and Genetics 8:309-314 which each describe other exemplary techniques from the art which would be recognized as means for generating mutagenic variants of CAK1 proteins.

It is plain from the combinatorial mutagenesis art that it was in fact routine for those skilled in the art to engage in large scale mutagenesis of proteins, without any preconceived ideas

of which residues were critical to the biological function, and generate wide arrays of variants having equivalent biological activity. Indeed, it is the ability of combinatorial techniques to screen billions of different variants by high throughout analysis that removes any requirement of a priori understanding or knowledge of critical residues. The only inventive step(s) required to utilize the nucleic acids as claimed have already been carried out by the Applicants. Routine screening techniques taught in the specification combined with those techniques available in the art at the time the present invention was made provide sufficient guidance for generating variants of the native CAK1 protein, as well as reducing the CAK1 proteins to minimum motifs. Accordingly, Applicants assert that the specification, in light of the art at the time the present invention was made, is enabling for a sufficient number of other permutations of the subject CAK1 proteins to entitle Applicants to the invention as presently claimed.

35 U.S.C. §112, first paragraph written description requirement

In subpart (b) of the Office Action, the Examiner states that "the claims encompass DNA encoding any polypeptide...including sequence variants and fragments of the disclosed polypeptide....(and encompass)...fragments or fragments with substitutions which are not functional polypeptide(s)." The Examiner concludes that "without such guidance, the experimentation necessary to determine all the possible variations of substitutions, modifications, or additions necessary to make a DNA encoding a functional CAK1 polypeptide is unpredictable."

For the reasons stated above, applicants respectfully traverse this element of the rejection as it relates to nucleic acids which encode polypeptides fragments that retain a CAK1 function or functions. Furthermore, the specification teaches that nucleic acids which encode mutant "dominant negative" polypeptide fragments of a gene may be readily obtained by high-throughput screening methodologies and used as competitive antagonists of the naturally-occurring gene (see pages 20-22 of the specification). Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

In subpart (c) of the Office Action, the Examiner further objects that "claim 15 requires an assay to determine an agonist or an antagonist of cell cycle regulation of a *Candida* cell" and that "the specification does not provide guidance nor a working example of an assay which utilizes

Candida cell". Applicants respectfully traverse this rejection. As discussed above, the specification provides numerous exemplary methods of screening for peptides and peptide fragments which retain wild-type CAK 1 activity or which antagonize (e.g. serve as a "dominant negative" antagonist) of CAK1 (see page 12, lines 22-32, and pages 21-22) by way of example with other *Candida* cell-cycle genes of the invention. For example, the CDK1-binding assays described for the *Candida* TYP1 phosphatase would be recognized by the skilled artisan as equally applicable to the *Candida* CAK1 kinase, which also recognizes and binds to the CDK1 kinase. Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

35 U.S.C. §112, second paragraph

Claims 14-22 and 37-40 have been rejected under 35 U.S.C. §112, second paragraph, as being "indefinite for failing to particularly point out and distinctly claim the subject matter which (the) applicant regards as the invention". In particular, the Examiner states that the term "homologous", "as the term is conventionally used in the art, is an evolutionary relationship of sequence comparisons whereas sequence 'identity' or 'similarity' refers to structural features of the sequence." Therefore, the Examiner concludes, the claims are "unclear as to what is the evolutionary significance and relationship between the CAK1 polypeptide and (the) SEQ ID No. 14." Applicants note that, as commonly used in the art of molecular biology, the word "homology" connotes both a quantifiable structural relatedness and an implied evolutionary relatedness. Furthermore, Applicants have defined "homology" as the sequence similarity between two peptides or between two nucleic acid molecules, which can be determined by aligning the sequences and determining when the amino acid or base at each position is the same - then the molecules are "homologous at that position" (page 11, lines 3-8). This use of the term "homology" is not repugnant to the normal meaning of the word when used in a molecular genetic context, and therefore meets the requirements of 35 U.S.C. §112, second paragraph. Nevertheless, in the interest of expediting prosecution of the patent and not in acquiescence to the rejection by the Examiner, Applicants have amended independent claim 14 and dependent claim 38 to recite "identical" rather than "homologous". As this amendment obviates the ground of rejection by the Examiner, reconsideration of the claims as amended and withdrawal of the rejection is respectfully requested.

The Examiner has further rejected claims 37-40 as indefinite for recitation of "stringent conditions, which is a relative term and it is not clear whether the condition is high, moderate, or low stringency condition for hybridization." Applicants respectfully traverse this rejection, because the term "stringent conditions" is supported in the specification and is well understood in the art to encompass conditions of hybridization which allow hybridization of structurally related, but not structurally dissimilar, nucleic acids. The term "stringent" is a term of art which is understood by the skilled artisan to describe any of a number of alternative hybridization and wash conditions which allow annealing of only highly complementary nucleic acids.

In particular, the specification describes stringent hybridization conditions as "equivalent to about 20-27°C below the melting temperature (T_m) of the DNA duplex formed in about 1M salt" (page 12, line 36 to page 13, line2). Many equivalent procedures exist and several popular molecular cloning manuals describe suitable conditions for stringent hybridization and, furthermore, provide formulas for calculating the length of hybrids expected to be stable under these conditions (see e.g. Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6 or 13.3.6; or pages 9.47-9.57 of Sambrook, et al. (1989) Molecular Cloning, 2nd ed., Cold Spring Harbor Press). Thus, the term "stringent" is not indefinite, but rather a functional description of many equivalent variations in salt and temperature conditions. Indeed it is well understood that there are many functionally equivalent methodologies for achieving stringent conditions. Each of these methodologies involves a number of interrelating variables such as the selection of hybridization membrane composition, hybridization buffer composition, hybridization buffer temperature, wash buffer composition, and wash buffer temperature. The skilled artisan recognizes that many of these factors interrelate. For example, the correct temperature for a stringent hybridization will depend upon the chemical composition of the hybridization buffer (68°C for aqueous buffers versus 42°C for buffers containing 50% formamide), and the selection of hybridization buffer conditions will depend upon the composition of the membrane used (nitrocellulose versus nylon) (see Sambrook, et al. (1989) Molecular Cloning, 2nd ed., Cold Spring Harbor Press). Therefore, Applicants respectfully submit that the metes and bounds of the claimed subject matter has been clearly set forth. Further, Applicants respectfully assert that, having defined a *Candida* CAK1 polypeptide-encoding gene they have enabled the further cloning of still other homologs in related fungi using any of a

number of equivalent stringent hybridization methodologies known in the art. Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

CONCLUSION

For the foregoing reasons, Applicants respectfully request reconsideration and withdrawal of the pending rejections. Applicants believe that the claims are now in condition for allowance and early notification to this effect is earnestly solicited.

If there are any other fees due in connection with the filing of this Response, please charge the fees to our Deposit Account No. 06-1448. If a fee is required for an extension of time under 37 C.F.R. § 1.136 not accounted for above, such an extension is requested and the fee should also be charged to our Deposit account.

Respectfully submitted,
FOLEY, HOAG, & ELIOT

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June 7, 2000

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